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Physicochemical, Biochemical and Genotoxic Profile of Subchronic Exposure of Wister Rats to Treated Crude Oil Exploration Water

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background

Crude oil exploration water is the major waste product generated from petroleum exploration and production activities and is known to be a complex composition of numerous hazardous chemicals, including large quantities of heavy metals, inorganic, and organic substances and naturally occurring radioactive materials (NORMs).

Objectives

This study was designed to investigate the physicochemical, biochemical and genotoxic profile of subchronic exposure of Wister rats to treated crude oil exploration water (TCOEW).

Materials and Methods

Fifty rats were randomly assigned to five treatment groups, with ten rats per group, and treated with five concentrations (1%, 5%, 10% and 20%) of TCOEW. Each TCOEW concentration was administered for 90 days *ad-libitum* as normal drinking water to each group, while the control group was given tap water. Blood, liver, kidney, thymus, spleen and femoral bone of the animals were collected at the end of exposure for biochemical and histological assessments. The pH, conductivity, and turbidity were carried out on-site, while other physicochemical parameters were determined using standard laboratory methods. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Results

Treated crude oil exploration water caused a significant ($p < 0.05$) increase in RBC, PCV and Hb while other hematological and biochemical parameters showed no difference. Also, TCOEW cause a significant increase in polychromatic erythrocytes (PCEs) in genotoxicity test and no major lesion were seen in the histopathology studies. Treated Crude Oil Exploration Water does not comply with FEPA standards of produced water due to high conductivity, salinity and total dissolved solid of the sample. The marked increase in micronuclei polychromatic erythrocyte formation (MNPCE) showed that TCOEW might be genotoxic and this could be responsible for the significant increase RBC, parked cell volume (PCV) and hemoglobin (Hb) observed.

Conclusion

Although TCOEW does not totally meet FEPA standard for effluent discharge, it has mild genotoxic effect.

Keywords: Physicochemical and genotoxic, Effluents treated crude oil exploration water, Flame Atomic Absorption Spectrometry

INTRODUCTION

Produced water still remains the largest operational source of oil pollution to the sea from offshore oil exploratory companies (Beyer *et al.,* 2020). Exploratory discharge of produced water and drilling cuttings from oil and gas production has become a major source of pollution to the biota of the Niger Delta region; increasing the health risks for rural communities that depend solely on the natural environment for sustenance and livelihood (Gazali *et al.,* 2017). Produced water is known to be a complex composition of numerous hazardous chemicals, including large quantities of heavy metals, inorganic, and organic substances, including naturally occurring radioactive materials (NORMs) (Clinton 2009; Andrade *et al.*, 2010). According to Tellez *et al.,* the two main disposal methods for produced water are environmentally unfriendly (Tellez *et al*., 2002). The array of hazardous chemicals contained in petroleum exploration waste streams and their unwholesome disposal has resulted in untold damage to environmental media that are unyielding to known remediation technologies (Yakubu, 2017).
Assessment and comparison of the physicochemical properties of produced water with the state's environmental protection agency's regulatory standards as a guide for processing exploratory wastes before discharge into the environment is essential. Usual practice involves treatment of the produced water with the sole aim of lowering the unwanted and hazardous component before reinjection into the water bodies (environment) or discharged into the sea (Jimenez *et al.,* 2018). Reinjection of exploratory produced water is considered the best environmental practice, and it is employed as a very common solution to management of offshore wastewater by oil exploratory companies (Beyer *et al.,* 2020).
With over 40 billion barrels of crude oil reserve in Nigeria's earth space, it is obvious that the continued global oil demand will require huge oil explorations and there are reasons to believe that the environmental risks will grow when new sources of oil are explored continuously (Zabbey and Olsson, 2017). Environmental Protection Agencies requires the treatment of the exploratory waste before disposal. The disposal of the large amount of produced water or Treated Crude Oil Exploration Water (TCOEW) into the water bodies would increase the likelihood of pollution and have tremendous effect on both the exposed animals and aquatic ecosystem, if ill-treated effluents are generated as various forms of waste water from oil exploratory industries. Decades of oil exploration in Niger Delta have caused unprecedented havoc to the region according to a 2011 environmental assessment report by United Nation Environment Program (UNEP). According to UNEP, locals of Nsisioken Ogale community in Ogoni land, a microcosm of Niger Delta, were reported to be drinking water with benzene level higher by 900 times above WHO recommendation (UNEP, 2016). Water pollution by heavy metal is associated with a number of gastrointestinal and kidney dysfunction, nervous system disorder, skin lesion, cancer, immune dysfunction, birth defects and vascular damages (Yajima *et al.*, 2015). Hence, the aim of this study is to compare the physicochemical properties TCOEW with the EPA standards and assess the sub-chronic toxicity profile of Treated Crude Oil Exploration Water (TCOEW) in Wistar rats.

METHODOLOGY

Materials and Method

Collection and storage of produced water

The produced water, TREATED CRUDE OIL EXPLORATION WATER was collected from the treatment plant of a crude oil exploring company in the Niger Delta area, transported to Department of Pharmacology and Therapeutics, University of Ibadan where it was stored between 2 - 8 °C in a refrigerator.

Experimental Animals

Male Wister rats weighing between 120 – 150 g were obtained from the central animal house, College of Medicine, University of Ibadan. The animals were housed in plastic cages and fed with standard rodent pellet feed and water *ad libitum* throughout the experimental period. They were acclimatized for at least 1 week prior to commencement of the experiments, thereafter; animals were *randomly* distributed according to experimental design. Fifty animals were divided into 5 groups (n = 10) and treated as follows; group 1 (water), groups 2, 3, 4 and 5 (1, 5, 10 and 20 produced water. Polyacrylate bottles (750 ml) fitted with metal snout attached to each cage were used to administered the different concentrations of the TCOEW. The groups were labelled as control, TCOEW 1, TCOEW 2, TCOEW 3 and TCOEW 4 respectively. All procedures in this study were performed in compliance with the National institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Ethical approval (UI-ACUREC/016-0120/29) was obtained from the Animal Care and Use Research Committee, University of Ibadan.

Subchronic toxicity studies

The OECD guideline (OECD 407) for sub-chronic toxicity was adhered to. The animals were provided TCOEW as drinking water *ad libitum* at 1%, 5%, 10% and 20% for 90 days. For the control group, distilled water was administered. The animals were sacrificed and blood samples for serum hematological and serum biochemical analysis were collected.

Physicochemicals analysis

On-site analyses of pH, conductivity, and turbidity were carried out at the site of sample collection following the standard protocols and methods of American Public Health Organization (APHA, 1995) and American Society for Testing and Materials (ASTM) using different calibrated standard instruments (DeZuane, 1997). The measurements of total suspended solids (TSS) and total dissolved solids (TDS) in TCOEW sample were carried out according to the standard methods of APHA, 1995 and Sawyer *et al.,* 1994 by the filtration process. Analyses of ten heavy metals such as Cu, Zn, Mg, Fe, Cd, Pb, Cr, As, Hg, and Sn were carried out based on ASTM standards (ASTM, 2000), which are approved by APHA using Flame Atomic Absorption Spectrometer (FAAS) (AAS, Perkin Elmer Analyst 400, available at Universiti Sains Malaysia, USM). For analysis of Cd, Cr, and Pb, direct extraction/air acetylene flame method was used, while manual hydride generation AAS method was used in determination of As (arsenic) in the samples. Cold-vapor AAS method was applied in the determination of Hg, with the aid of air-acetylene flame.

Biochemical analysis

Serum chemistry

The serum biochemistry was assessed using Synchron Clinical System CX4 (Beckman Coulter, Brea, CA USA) according to the manufacturer's directions (Beijing Leadman Biochemistry Technology Co. Ltd, Beijing, China). In serum biochemistry analysis, parameters measured include alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), glucose (GLU), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine (CREA), sodium (Na) and Chloride (Cl).

Hematological analysis

Hematological measurements and calculations were performed by using Coulter HmX Hematology Analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Hematological evaluations included red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell volume distribution (RDW), blood platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and white blood cell count (WBC).

Mammalian Bone Marrow Micronucleus Assay

Animals were sacrificed by cervical dislocation and (Schmid, 1975; Bakare *et al.,* 2009) the bone marrow was flushed into Eppendorf tubes using 0.5 ml of Fetal Bovine Serum (FBS). The cells were centrifuged at 2000 g for 5 min and smear made on pre-cleaned grease free slides. Slides were air dried and stained with May–Grunwald and Giemsa stains. They were coded and examined under an Olympus light microscope at 1000× magnification. 2000 cells per rat were scored for micronucleated polychromatic erythrocyte (MNPCE).

Statistical analysis

The data were expressed as Mean ± S.E.M. (standard error of mean). The data was analyzed using Kruskal–Wallis test (Non-parametric) and one–way analysis of variance (ANOVA) followed by post–hoc test (Dunnet's test) for multiple comparisons where appropriate using Graph Pad Prism software version 5. A level of $p < 0.05$ was considered as statistically significant for all tests.

RESULTS

Physicochemical analysis of TCOEW

The table below shows some physical and chemical parameters of TCOEW in comparison with Federal Environmental Protection Agency (Table 1).

Table 1: Comparison of the measured physicochemical parameters of TCOEW with FEPA standards

PARAMETER	TCOEW	FEPA
pH	6.81	6.5-8.5
Temperature (°C)	19.1	35
Dissolved Oxygen (mg/l)	6.23	5.0
Conductivity (µs/cm)	19,700	250
Total Dissolved Solid (mg/l)	9,850	30
Salinity (%)	6,532	-
Total Suspended Solid (mg/l)	37.0	30
Biochemical Oxygen Demand (mg/l)	7.76	10
Turbidity (NTU)	5.0	30
Total Petroleum Hydrocarbon	0.134	10.00
Polycyclic Aromatic Hydrocarbon	0.038	0.00
Cadmium (mg/L)	0.102	<0.5
Zinc(mg/L)	0.090	1.0
Lead(mg/L)	0.041	0.05
Chromium(mg/L)	0.112	0.5
Copper(mg/L)	0.124	1.5
Iron(mg/L)	0.221	1.00
Mercury(mg/L)	<0.001	-
Nickel(mg/L)	0.061	-
Arsenic(mg/L)	<0.002	-
Barium(mg/L)	0.092	-
Cobalt(mg/L)	0.064	-

TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on serum biochemistry of Wister Rats

The results of serum biomarkers (ALT, ALP, AST, BUN, creatinine etc. as shown in Table 2) measured in the TCOEW treated groups, did not show any significant alteration in comparison with the control group treated distilled water. Total bilirubin (mg/dl) and direct bilirubin (mg/dl), however were significantly higher in the TCOEW treated groups in comparison with the control group.

Table 2: Effect of treatment with TCOEW on serum biochemistry of Wister rats

PARAMETERS	H_2O	TCOEW 1%	TCOEW 5%	TCOEW 10%	TCOEW 20%
AST(U/L)	42±0.91	40.25±1.54	39±1.58	40.75±1.25	40±1.29
ALT(U/L)	30.5±1.04	29±1.41	28.5±1.19	28.75±1.49	28.75±1.55
ALP(U/L)	116.3±4.01	99.75±8.51	91.5±6.58	94.75±8.18	93.75±8.51
BUN (mg/dl)	16.35±0.35	17.03±0.60	16.45±0.35	16.85±0.33	16.4±0.72
Creatinine (mg/dl)	0.575±0.03	0.6±0.06	0.575±0.03	0.65±0.03	0.6±0.04
Total Bilirubin (mg/dl)	0.225±0.05	0.55±0.05*	0.35±0.05*	0.25±0.06	0.325±0.10*
Direct Bilirubin (mg/dl)	0.00575±0.001	0.035±0.09*	0.02±0.004*	0.035±0.009*	0.0375±0.005*
GLUCOSE (mg/dl)	140.5±2.1	138.8±0.75	141.3±1.84	141.8±1.31	143.8±1.65
Cholesterol (mg/dl)	46±2.38	41.75±2.01	41.75±1.84	41.75±1.03	43.5±2.32
Triglyceride (mg/dl)	462±2.38	41.75±2.01	41.75±1.84	41.75±1.03	43.5±2.32
HDL (mg/dl)	38.5±2.96	34.75±2.01	35.5±1.84	37.25±0.47	35.5±5.39
Na (Meq/L)	143.5±1.19	144±0.71	139.3±1.25	143±1.73	140.8±1.931
Cl (Meq/L)	108.3±2.96	109.3±2.49	106.3±2.92	108±4.06	111.3±2.28

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test. * = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water; TCOEW = Treated Crude Oil Exploration Water; AST = Aspartate aminotransferase; ALT= Alanine aminotransferase; ALP = Alkaline phosphatise; HDL = High density lipoprotein; Na^+ = Sodium; Cl^- = Chloride

Effect of TCOEW on haematological profile of Wister rats

Treatment with TCOEW significantly increased PCV, Hb and RBC in comparison with control treated with water. Haematological parameters such as WBC, platelets, lymphocytes, monocytes, eosinophil, etc., were not significantly altered by treatment with TCOEW in comparison with the control. (Table 3). There was a significant reduction in neutrophil (%) at 40% treatment with TCOEW. in comparison with the control group.

Table 3: Effect of TCOEW on haematological profile of Wister rats

PARAMETERS	H_2O	TCOEW1%	TCOEW5%	TCOEW10%	TCOEW20%
PCV (%)	42.75±0.85	50±2.97	52±2.21	53.75±2.87*	55.5±3.30**
Hb (g/Dl)	14.3±0.36	16.5±0.72*	17.28±0.69*	17.15±0.58*	18.03±1.00*
RBC $\times 10^6$ U/L	7.12±0.09	8.215±0.31*	8.475±0.15*	8.435±0.29*	8.743±0.14*
WBC$\times 10^3$ U/L	5963±1670	NA	4150±926	4438±310.50	5450±670.2
Platelet ($Ul^{-1)}$	122000±2549	110250±145	133750±217	144250±7307	157750±126
Lymphocyte (%)	73.5±0.64	76.25±1.11	75.25±1.03	76±0.70	76.75±1.11
Neutrophil (%)	24±0.81	21.5±1.19	23.25±1.10	21.75±0.47	19.5±0.86*
Monocytes (%)	1.5±0.28	1±0.00	1.25±0.25	1.5±0.28	1.5±0.28
Eosinophil (%)	1±0.41	1±0.00	0.25±0.25	0.75±0.25	2.25±0.47
Protein (%)	6.9±0.21	7.35±0.40	7.225±0.39	7.675±0.39	7.075±0.56
ALBUMIN (G/Dl)	2.95±0.14	2.875±0.10	2.875±0.14	3.2±0.11	3.075±0.22
GLOBULIN (G/Dl)	3.95±0.09	4.475±0.31	4.3±0.17	4.475±0.29	4±0.39
A/G Ratio	0.7±0.04*	0.55±0.03	0.625±0.03	0.675±0.03	0.725±0.06

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's multiple comparison test. * = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water; TCOEW = Treated Crude Oil Exploration Water; RBC: red blood cell, PCV: Packed cell volume; Hb; Hemoglobin; A/G Ratio: Albumin/Globulin Ratio

Effect of TCOEW on micronuclei polychromatic erythrocyte formation in Wister rats

TCOEW treated group-TCOEW 5%, TCOEW10% and 20% showed increase in micronuclei polychromatic erythrocyte formation (MNPCE) which was significant (p <0.05) in TCOEW 5% and TCOEW20%

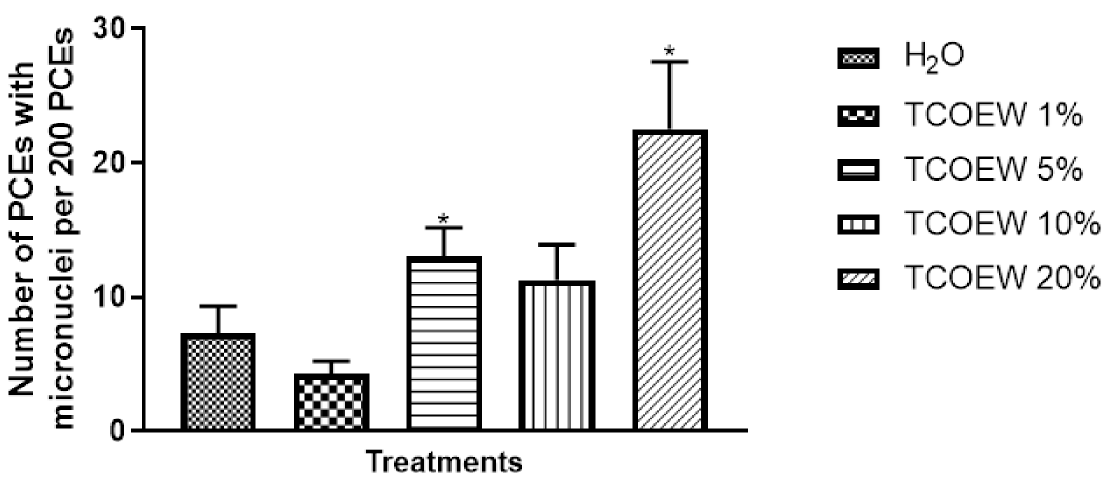

Figure 1: Effect of TCOEW on micronuclei polychromatic erythrocyte formation (MNPCE) in Wister rats

All values were expressed as mean ± SEM (n = 6). Data were analysed using one-way ANOVA followed by Dunnet's *post-hoc* test.* = significance at $p < 0.05$ when compared with H_2O only. H_2O = Tap water. TCOEW = Treated Crude Oil Exploration Water

Effect of TCOEW on kidney histology of treated Male Waster Rats

TCOEW1%: The glomeruli (star) and tubules (black arrows) appear fairly normal. No visible lesion.

TCOEW5%: The glomeruli (star) appear normal. There are multiple foci of flattened tubular epithelial cells (blue arrows). There is moderate congestion of interstitial renal blood vessels (red arrow)

TCOEW10%: The glomeruli (star) appear fairly normal. There are numerous foci of cloudy swelling and degeneration of the tubular epithelial cells (black arrows).

TCOEW20%: The glomeruli (star) appear fairly normal. There are multiple foci of cloudy swelling and degeneration of the tubular epithelial cells (black arrows).There is mild congestion of the renal blood vessels and glomerular capillaries (red arrows)

H_2O (Control): The glomeruli (star) and tubules (black arrows)

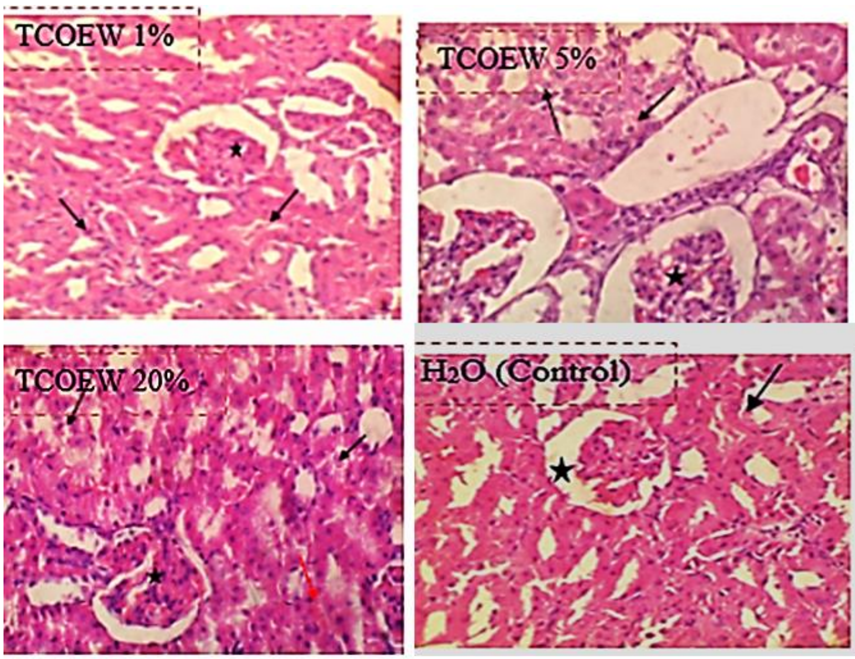

Figure 2: Effect of TCOEW on the kidney of treated male Wister rats (Magnification: X100; TCOEW: Treated Crude Oil Exploration Water)

Effect of TCOEW on liver histology of treated male Wister rat

TCOEW 1%: Hepatic plates/cords are closely-packed. Hepatocytes (black arrows) generally have finely reticulated cytoplasmic appearance. Vascular changes are not remarkable. There is mild Kupffer cell hyperplasia (green arrows). There are a few foci of single-cell hepatocellular necrosis (black arrows).

TCOEW 5%: Similar to TCOEW1%

TCOEW 10%: Hepatic plates/cords are closely-packed. Hepatocytes (black arrows) generally have finely reticulated cytoplasmic appearance. Vascular changes are not remarkable. There is mild Kupffer cell hyperplasia (green arrows). There are a few foci of single-cell hepatocellular necrosis (black arrows).

TCOEW 20%: Similar to TCOEW10%

H_2O (Control): closely-packed. Hepatocytes (black arrows) and Kupffer cell (green arrows)

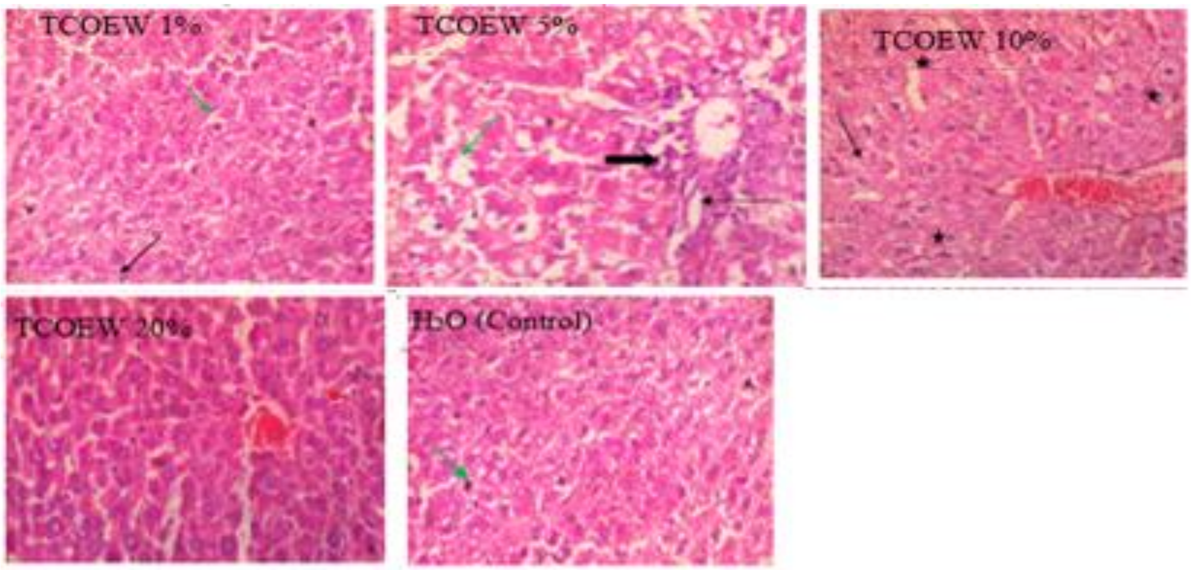

Figure 3: Effect of TCOEW on Liver histology of treated male Wister rats (Magnification: X100; TCOEW: Treated Crude Oil Exploration Water)

Effect of TCOEW on Spleen histology of treated Male Waster Rats

TCOEW1%: There are moderate amounts of discrete Periarteriolar Lymphoid sheet-PALSs (black arrows) with large germinal centres (stars). There is moderate congestion of the splenic sinusoids (red arrows).

TCOEW5%: There are discrete PALSs (stars). There is moderate congestion of the splenic sinusoids (black arrows).

TCOEW10%: There are discrete small PALSs (stars). There is marked congestion of the splenic sinusoids and sinuses (red arrows).

TCOEW20%: There are moderate numbers of discrete PALSs (stars). There is moderate congestion of the splenic sinusoids (black arrows).

H_2O (Control): Splenic sinusoids (red arrows) and germinal centres (stars)

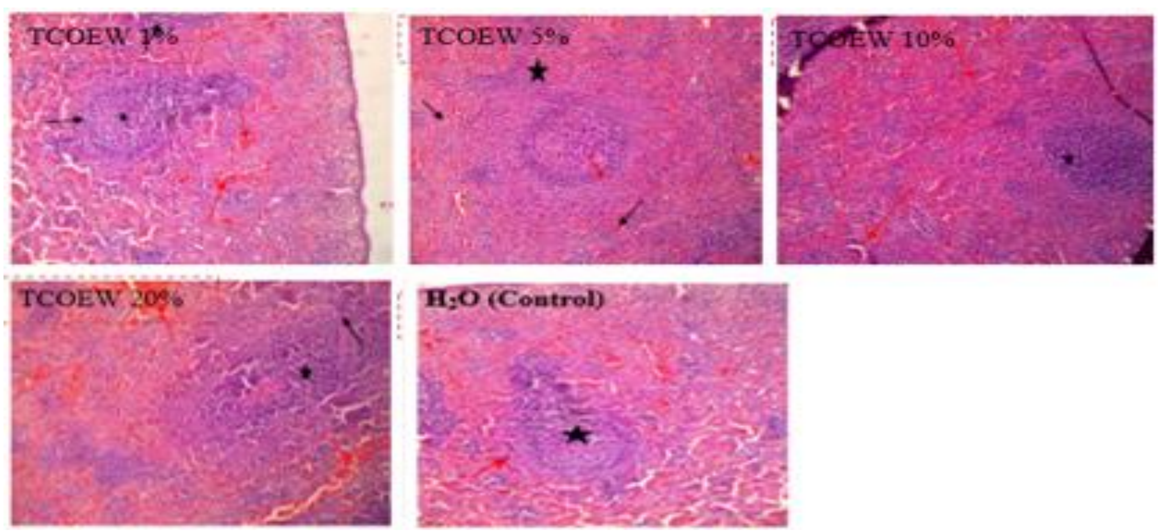

Figure 4: Effect of TCOEW on Spleen histology of treated Male Wister Rats (Magnification: X100; TCOEW: Treated Crude Oil Exploration Water)

Effect of TCOEW on Thymus histology of treated Male Waster Rats

TCOEW 1%: The ratio of the thymic cortex (black arrow) to the medulla (star) is reduced

TCOEW 5%: The ratio of the thymic cortex (black arrow) to the medulla (star) is reduced. There is moderate congestion of the blood vessels (red arrow)

TCOEW 10%: The thymic cortex (black arrow) and medulla (star) appear fairly normal. There is mild congestion of the blood vessels (red arrow)

TCOEW 20%: There is marked involution of the thymus with infiltration of adipose tissue (thick arrow). The capsule is wrinkled (green arrow).The remnant of the thymus has dense cortical aggregates of lymphocytes (black arrow). There is mild congestion of the blood vessels (red arrow)

H_2O (Control): The ratio of the thymic cortex (black arrow) to the medulla (star) is normal

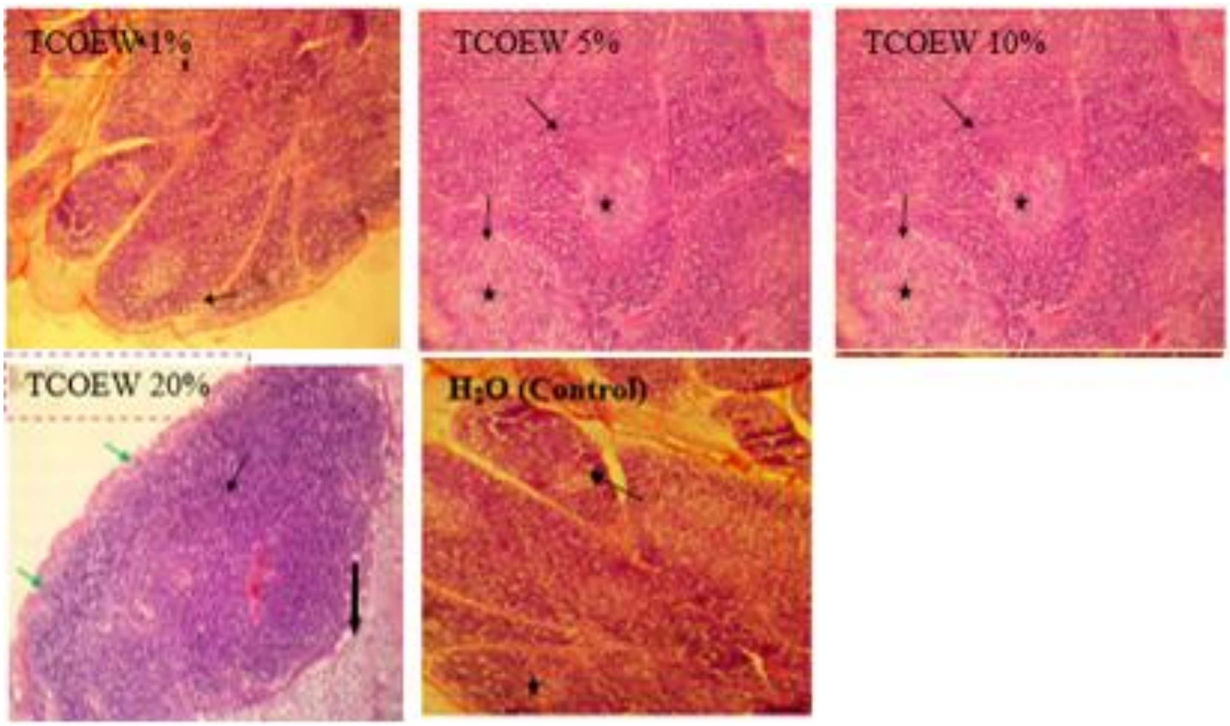

Figure 5: Effect of TCOEW on Thymus histology of treated Male Wister Rats (Magnification: X100; TCOEW: Treated Crude Oil Exploration Water)

DISCUSSION

Nigeria was the first African country to establish a national institutional mechanism for environmental protection. The Federal Environmental Protection agency (FEPA) established in 1988, which was charged with the overall responsibility for environmental management and protection (Obasi, 2019). The FEPA created guidelines for permissible limits of different industrial toxicants that could be released into the environment including waste water from crude oil exploration also called produced water. Physical and chemical parameters of TCOEW collected were analyzed using standard methods for waste water analysis. Treated Crude Oil Exploratory Water appeared as a dirty brown coloured water with a slight odour. The physicochemical analysis of TCOEW shows that the effluent does not comply with FEPA standards of produced water with very high conductivity, salinity and total dissolved solid. High conductivity does not directly have any health implication but the dissolved ionisable solids have effect on the taste and overall satiability of the water. The recorded value points at the fact that TCOEW contains lots of chemicals and impurities. Conductivity, total dissolved solid and salinity are far beyond the FEPA range. Biochemical oxygen demand (BOD), turbidity, total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) values are than FEPA standard. A lower BOD bodes signifies that less oxygen is removed from the water, implying that it has a good quality (D'Angelo *et al*., 2015). The toxic oil components are estimated by PAH, TPH estimates the concentration of higher molecular weight hydrocarbon (Abassi and Kesharvazi, 2019).

Alteration in serum biochemistry has been used to monitor liver and kidney functions which are highly essential for normal functioning and survival of the animals. Biomarkers for liver and kidney functions indexes were in the normal ranges after 90 days exposure to TCOEW. The variation in ions which depicts heart, muscle and neural functions were also in the normal range. However, the increased direct and indirect bilirubin levels in the serum is a sign that the liver is not clearing bilirubin properly which might be an indication of a liver disease. A common and harmless cause of elevated indirect bilirubin is Gilbert's syndrome which is usually as a result of destruction of too many blood cells (Bulmer et al., 2018). Hematological parameters are also use as assessment tool for detecting toxicity (Nigatu *et al.*, 2016). TCOEW treated group showed significant changes in hematological parameters i.e. RBC, parked cell volume (PCV) and haemoglobin (Hb) were markedly increased while other hematological parameters such as lymphocytes, platelets, neutrophils, monocytes, eosinophils, albumin and globulin all showed no significant change. Increased PCV is most commonly caused by dehydration resulting too many red blood cells and eventual thickening of the blood (CDC, 2013). Red blood cells account for nearly all the cells in the blood. The PCV increases when the number of red blood cells increases or when the total blood volume is reduced, as in dehydration. This result correlates with the elevated bilirubin.

Micronuclei (MN) are formed in addition to the main nucleus in cells as a result of acentric fragments or lagging chromosomes that failed to incorporate into either of the daughter nuclei during cell division (Adeoye *et al.,* 2015). Micronuclei test is the most widely utilized test for the genotoxic and mutagenic assessment of xenobiotics due to its technical simplicity, less time consumption and ability to detect both clastogens and aneugens (Krishna and Hayashi, 2000). TCOEW treatment showed increase in micronuclei polychromatic erythrocyte (MNPCE) formation implying it ability to induce chromosome damage and indicating that it might be genotoxic,

The representative histological images obtained from similar anatomic locations in the kidney, liver, spleen and thymus showed no obvious implicative tissue structure changes. Although, a small number of sporadic lesions were observed in some treatment and control groups, the macroscopic and microscopic inspection of the animals in the treatment and control groups did not show any significant histopathologic changes attributable to the exposure to TCOEW.

CONCLUSION

The Treated Crude Oil Exploration Water did not comply with FEPA standards of produced water due to high conductivity, salinity and total dissolved solid of the sample. The marked increase in micronuclei polychromatic erythrocyte formation (MNPCE) showed that TCOEW might be genotoxic and this could be responsible for the significant increase RBC, parked cell volume (PCV) and hemoglobin (Hb) observed. This study provides evidence that treated crude oil exploration water might contain clastogens and aneugens which on chronic exposure result in genotoxicity. Further studieswill be needed to ascertain the specific compounds responsible for these effect. The study thus recommends strict compliance with the international standards for treatment of produced waste water before discharge into the environment.

ETHICAL CONSIDERATIONS

Ethical approval (UI-ACUREC/016-0120/29) was obtained from the Animal Care and Use Research Committee, University of Ibadan.

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